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Datasheet for K-025

NFkB Oligonucleotide

Overview

Description:	NFkB Oligonucleotide - K-025
Item No.:	K-025
Size:	500 ng

Product Details

Background:	NFkB oligonucleotide consensus sequence for binding of NFkB /c-Rel homodomeric and heterodimeric complexes. Supplied as 500 ng of double stranded DNA for gel shift and super shift assays. In general, NFkB gel shift assays are assembled in 20µl reactions containing 0.28pmoles NFkB oligo in 10mM Tris (pH 7.6), 50 mM NaCl, 0.5 mM EDTA, 1.0 mM DTT, 10% glycerol. Some procedures specify the addition of 0.05% NP-40. When using purified protein, 250-300 ng should be sufficient to produce a gel shifted complex, while 10µg HeLa nuclear extract is utilized. The gel shift reactions are then incubated at room temperature for 30 minutes. The complexes are resolved on a Tris-Glycine acrylamide gels. Loading dye containing bromophenol blue and xylene cyanol should be added to the negative control reaction only, as these dyes can increase the dissociation of the NFkB complexes. When using HeLa nuclear extract as the source of binding proteins, two sequence-specific gel-shifted complexes are expected, consisting of p50/p50 homodimers and p50/p65 heterodimers. For cells expressing p52, p50, and p65, as many as four sequence-specific gel-shifted complexes could be observed (p52/p52, p50/p50, p52/p65, p50/p65), and if high levels of p65 are present, the p65/p65 homodimer may also be weakly detected. The following reagents have been observed to enhance NF-kB binding in vitro: millimolar amounts of GTP and ATP, spermine, spermidine, barium or calcium ions, and µM amounts of Co+3(NH3)6.
Synonyms:	NFkB NFkappaB

Target Details

Purity/Specificity:	Consensus sequence AGTTGA-G-G-G-G-A-C-T-T-T-C-C-CAGGC (top strand only KB consensus site separated by ''-'').

Application Details

Application Note:	Gel Shift/Gel Super Shift Assays: the electrophoretic gel shift assay is used to detect sequence

specific DNA-binding proteins present in nuclear extracts. For NFkB generally a HeLa nuclear extract is used. In the assay, a consensus oligonucleotide is end-labeled with isotopic phosphorus and detected using autoradiography. Other non-radioactive methods have also been employed including chemiluminescence, fluorescence and enzymatic assays. A 'gel shift' of radiolabel is observed whenever the DNA binding protein forms a complex with radiolabeled oligonucleotide resulting in the detectable label migrating at a higher apparent molecular weight. The 'gel super shift' assay refers to the additional increase in apparent molecular weight resulting from binding a specific antibody to the DNA binding protein prior to reaction with radioactive probe. Hence through the use of a specific antibody and a consensus oligonucleotide the researcher can identify the presence of a specific DNA binding protein in any nuclear extract.

Reagents Required:

1. Molecular Biology Grade Water.
2. Poly d(I) d(C). Use this as a non-specific inhibitor.
3. 10X TGE Buffer. Prepare a 10X concentrate of Tris-Glycine-EDTA (TGE) by adding 30.3 g Tris Cl, 142 g glycine, 37.2 g EDTA and deionized water to a final volume of 1.0 liter. Do not adjust the pH.
4. 5% TGE Gel. Prepare 60 ml of solution by mixing 10.5 ml 30% polyacrylamide, 6 ml 10X TGE, 3 ml glycerol, 40 ml H_2O, 0.45 ml 10% ammonium persulfate and 0.06 ml TEMED.
5. 5X NFkB Binding Buffer. This 5X concentrated buffer is composed of 250 mM NaCl, 50 mM Tris Cl, 50% (v/v) glycerol, 5 mM DTT, 2.5 mM EDTA adjusted to pH 7.6. Store this buffer prior to use at -20° C.
6. Nuclear Extract. Prepared from a cell line known to be positive for DNA binding protein (i.e. HeLa for NFkB).
7. 32P-labeled DNA probe. Add 30,000-50,000 CPM double stranded consensus oligonucleotide probe per reaction mixture. For NFkB the consensus sequence is GGGGACTTTCC. As a control also prepare probe without label (cold).
8. Super Shift Antibody. Add recommended volume of antibody specific for DNA binding protein (usually 1 µL).

Protocol:

1. Add the following to a microfuge tube (the volume of H_2O added should result in a total reaction volume of 20 µL including the labeled probe): poly dI-dC to 2 µg/rxn, 4 µL 5X Binding Buffer, 2-5 µg Nuclear Extract and x µL H_2O.

2. Gently mix the contents of the tube.

3. For the supershift assay add the antibody to the reaction mixture and incubate the reaction for 15 min at room temperature. Omit this step if only performing the gel shift assay.

4. Add the 32P labeled probe and gently mix. Incubate the reaction for 15 min at room temperature.

5. Load the entire reaction mixture volume into each lane of a 5% polyacrylamide gel (1.5 mm x

	20 cm x 20 cm) prepared in TGE buffer. Do not add dye to the reaction mixture lane. Dye may interfere with binding. Run the dye separately in the first and last lanes of the gel. 6. Run the gel at 20 milliamps for 1.5 to 2 h. Dry the gel and perform autoradiography to visualize banding patterns. Notes: For best results let the gel polymerize for 1h then pre-run the gel for 1 h using a constant current of ~20 milliamps. Typically 2 liters of 1X TGE is used: 1.5 liters in the bottom reservoir and 0.5 liters in the top reservoir when using a commercially available apparatus. Do not exceed a final concentration of 100 mM sodium chloride in the reaction mixture. Concentrations above 100 mM inhibit the reaction. Do not exceed 2.5 µL of nuclear extract per reaction mixture. This is a good generalized method. Specific antibodies/probes may require altered conditions. Prepare the reaction mixture in duplicate using 1-10 ng of unlabeled (cold) probe as a negative control or add cold probe and incubate 10 min at room temperature before adding labeled probe for competition experiments. Certain gel super shift antibodies are supplied with control peptides. Prepare these reaction mixtures in duplicate adding the control peptide to the reaction mixture prior to adding the antibody.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:5,000 - 1:25,000
EMSA:	See protocol
WB:	1:500 - 1:3,000

Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	20 ng/µL by UV absorbance at 260 nm
Buffer:	0.01 M Tris Cl, 0.15 M Sodium Chloride, 0.001 M EDTA, pH 7.4
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

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